Check for updates

OPEN ACCESS

DOI: 10.46903/gjms/21.03.1285

ORIGINAL ARTICLE

COMPARISON OF PROTEIN C INHIBITOR (PCI) BLOOD LEVELS IN WOMEN BEFORE STARTING SECOND GENERATION ORAL CONTRACEPTIVE PILLS AND AFTER THREE MONTHS OF USE

Namra Mahmood

Department of Pathology, Central Park Medical College, Lahore, Pakistan

ABSTRACT

Background: Protein C inhibitor (PCI) is a serine protease inhibitor synthesized by the liver. Apart from its function of inhibiting activated protein C (APC), PCI also inhibits activated factor II, thrombin thrombomodulin complex, factor XIa, tissue plasminogen activator (t-PA) and urokinase plasminogen activator (u-PA). Increased level of PCI has been found in patients with thrombosis and women using oral contraceptives. The aim of the present study is to observe the effect of combined oral contraceptive pills on PCI levels after three months of its use and mean change from baseline.

Materials & Methods: Cross sectional descriptive study carried out at the pathology department, post graduate medical institute (PGMI) between 01/03/2018 to 02/03/2019 after taking ethical approval. Purposive convenience sampling technique was used. Blood sample of the study population was taken before the start of medication and after three menstrual cycles. Protein C inhibitor levels and demographic data was collected and analyzed using SPSS.

Results: Among total 54 females, the age of the women ranged from 22 years to 44 years with a mean of 29.5 ±5.3 years. The parity of the women ranged from 1 to 6 with a mean of 3.06 ±1.20. The baseline value of protein C inhibitor levels ranged from 1.1 to 8.7 mg/l with a mean of 5.84 ± 1.83 mg/l at baseline and increased upon 3-months follow-up to 8.2 to 20.1 mg/l with a mean of 12.74 ±3.29 mg/l. The women aged 29-35 years had highest of PCI levels at follow up with a mean of 13.47 ±4.28 mg/l and primiparas had highest increase among groups and it was statistically significant (p-value < 0.001).

Conclusion: It can be concluded from the study that use of the second generation oral contraceptive for 3 months is associated with induction of prothrombotic state by increase in the level of protein C inhibitor in the absence of any other prothrombotic conditions.

Keywords: Activated Protein C inhibitor; Protein C inhibitor; Thrombosis; Deep vein thrombosis; Oral contraceptives; Contraceptive agents.

Cite as: Mahmood N. Comparison of protein C inhibitor (PCI) blood levels in women before starting second generation oral contraceptive pills and after three months of use. Gomal J Med Sci 2023 Jul-Sep;21(3):141-4. https://doi.org/1046903/gjms/21.03.1285

INTRODUCTION

The accurate functioning of the protein C anticoagulant pathway is important and is illustrated by the fact that any flaws in this pathway are linked to an increased risk of venous thromboembolism. Inadequate amounts or improper functioning of protein C or protein S, if present, incite unchecked generation of thrombin and uncontrolled clotting process which results in obstructed blood flow in the vessels.[1] In blood plasma, an inhibitor of activated protein C (APC) is present which is a component of this anticoagulant pathway and belongs to a group of serine protease inhibitors known as protein C inhibitor (PCI) or plasminogen activator inhibitor-3.[2] It is a glycoprotein and a heparin-binding serpin, which is synthesized by the liver. Apart from its function of inhibiting APC, PCI also inhibits activated factor II, thrombin thrombomodulin complex, factor XIa, tissue plasminogen activator (t-PA) and urokinase plasminogen activator (u-PA).[3,4] In the absence of thrombomodulin, PCI is only a poor inhibitor of coagulation. The importance of PCI as an

Corresponding Author:

Dr. Namra Mahmood
Assisstant Professor, Department of Pathology
Central Park Medical College, Lahore, Pakistan
E-mail: dr.namra.mahmood@gmail.com

Date Submitted: 26-01-2023
Date Revised: 12-07-2023
Date Accepted: 20-08-2023

essential regulator maintaining equilibrium between coagulation and fibrinolysis lies in the fact that it arrests the activation of thrombin-activatable fibrinolysis inhibitor (TAFI) as well as inhibition of activated protein C.[2,4]

Meijers et al. determined protein C inhibitor (PCI) plasma levels in patients at the time of thrombotic occurrence who presented with deep venous thrombosis. A fall in PCI plasma levels was identified and an additional fall in levels was noticed when heparin was started as a treatment.[5] Similarly, Tans et al. and Bremme et al. identified a significant raise in protein C inhibitor plasma levels with desogestrel-containing oral contraceptive use.[6,7] Various coagulation factors, fibrinolytic factors and their physiological inhibitors have been investigated with the oral contraceptives use and have shown to cause notable alterations in numerous hemostatic variables.[8] Several present-day studies have also emphasized the decreased risk of thromboembolic events in combined oral contraceptive (COC) users with decreasing dose of estrogen as well as its short duration of use.[9] Prospective researches have also demonstrated the relationship between the COC pill and venous thromboembolism (VTE). A large cohort study was conducted in 1974 which studied two cohorts of around 23,000 subjects each and recorded a standardized relative risk of VTE of 4.2.[10]

Other prospective cohort studies such as The Walnut Creek Study, The Oxford Family Planning Study and The Seattle Health Maintenance Organization Study reached similar conclusions with regard to estimates of risk. All these studies related to oral contraceptive pill and its risk of VTE were considered and reviewed by Koster and associates who concluded that the use of the oral contraceptive pill conferred around a three-fold rise in risk of venous thrombosis. The surge in risk developed immediately on starting the oral contraceptive pill, although it was not related to time-span of use. The risk disappeared following discontinuation of the pill.[11] The aim of the present study is to observe the effect of second generation oral contraceptive pills on activated protein C inhibitor levels in blood before starting and after three months of its use in reference of age and parity groups and its mean change from baseline. As studies on this subject are lacking in the local population, this study targets to fetch information about our population response to COCs and its effect on coagulation system particularly on protein C inhibitor levels.

MATERIALS AND METHODS

This was a cross-sectional descriptive study carried out at the Pathology department of post graduate medical institute (PGMI)/Lahore General Hospital, Lahore. It was a prospective study conducted between 01/03/2018 to 02/03/2019 after taking ethical approval. Purposive convenience sampling technique was used.

Sample size was calculated using WHO sample size calculator 2.0. Fifty four (54) female patients aged between 18-45 years who were scheduled by the family planning clinic of Lahore General Hospital, Lahore to start second generation oral contraceptive pills (Levonorgestrel 150 µg and Ethinyl Oestradiol 30 µg tablets USP) were included in the study. Second generation oral contraceptive pill is available by the name Famila 28F in public sector hospitals of Punjab, Pakistan. Females between ages 18-45 years were included in the study that had not used hormonal contraceptives for the last three months. Females with hemophilia, venous or arterial thrombotic tendencies, taking oral anticoagulants and those having metabolic or chronic illness were excluded.

For each patient, after obtaining informed consent, data was entered in the predesigned proforma. All included patients used same medicines for three consecutive months in a standard way. Blood sample of the study population was taken twice, first before the start of medication (baseline) and second at the end of three months (follow-up). 3ml blood sample was collected in a blue capped vacutainer tube (having 3.2% sodium citrate anticoagulant) aseptically. After centrifugation of the sample for 15 minutes at 2500 rpm, supernatant was saved in separate tubes and stored at -80Ċ. The samples were processed for protein C inhibitor by ELISA method in Pathology department, Lahore General Hospital, Lahore when the sample collection from all the patients was completed.

Data was analyzed by using SPSS version 21.0. Numerical variables: age, protein C inhibitor levels at baseline and at follow-up were presented by mean ±SD. Paired sample t-test was used to observe the difference between baseline and follow-up observations taking p-value ≤0.05 as statistically significant. Categorical variable; like, parity was presented as frequency and percentage. Data was stratified for age and parity to address effect modifiers. Poststratification, paired sample t-test was applied to compare baseline and follow-up observations across each strata taking p-value ≤0.05 as statistically significant.

RESULTS

Age distribution of the study group

Among total 54 females, the age of the women ranged from 22 years to 44 years with a mean of 29.5±5.3 years as shown in Figure 1.

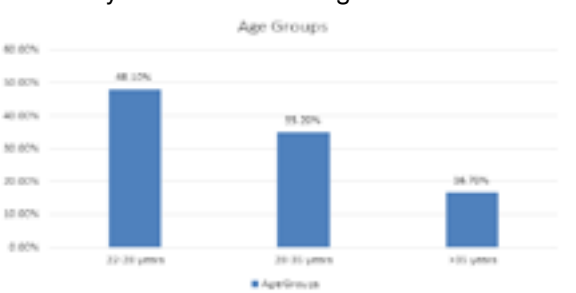

Figure 1: Age group distribution in study population

Table1: Protein C inhibitor levels across different age groups.

Age	At Baseline (mg/l)	At Follow up (mg/l)	Mean Change (mg/l)	P-value
22-28 years	6.20±1.78	12.62±2.75	6.43±2.58	<0.001*
29-35 years	5.70±1.88	13.47±4.28	7.77±3.38	<0.001*
>35 years	5.13±1.79	11.52±1.97	6.39±2.06	<0.001*

*Paired sample t-test, observed difference was statistically significant

Table No.2: Protein C inhibitor levels across different parity groups.

Parity	At Baseline (mg/l)	At Follow up (mg/l)	Mean Change (mg/l)	P-value
Primiparas	6.11±1.51	13.47±2.13	7.36±2.72	<0.001*
Multiparas	5.75±1.93	12.82±3.49	7.08±2.86	<0.001*
Grand Multiparas	6.43±1.28	10.53±1.98	4.10±1.50	0.012*

*Paired sample t-test, observed difference was statistically significant

Parity distribution of the study group:

The parity of the women ranged from 1 to 6 with a mean of 3.06±1.20 as shown in Figure 2.

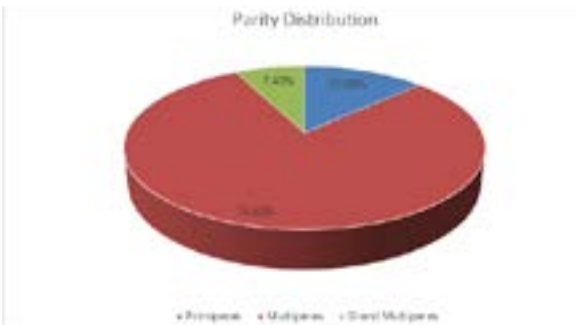

Figure 2: Parity groups of the study population

Baseline values across the study group

The values of protein C inhibitor ranged from 1.1 to 8.7 mg/l with a mean of 5.84±1.83 mg/l at baseline. The protein C inhibitor levels increased upon follow-up and ranged from 8.2 to 20.1 mg/l with a mean of 12.74±3.29 mg/l. The mean increase in PCI from baseline to follow-up was 6.89±2.84 mg/l and was statistically significant (p-value <0.001).

Values across various age groups

Protein C inhibitor levels across various age groups are shown in Table-1.

Values across various parity groups

Protein C inhibitor levels across various parity groups are shown in Table-2.

DISCUSSION

Fifty four (54) women were studied in this research who were planned to receive oral contraceptives, the levels of protein C inhibitor upon follow up significantly with a mean change of 6.89±2.84 mg/l (p-value<0.001). Similar change was observed across various subgroups based on patient's age and parity. Thus, oral contraceptive use led to suppression of anti-coagulation pathway leading to subsequent unchecked coagulation pathway and hypercoagulable state which explains the DVT and VTE associated with their use.

Kemmeren et al. in a similar study in Netherlands reported that protein C inhibitor levels were significantly higher in women receiving second generation oral contraceptives as compared to controls (101.0±15.0 vs. 90.6±16.6; p-value<0.001). They too concluded that inhibition of protein C was the probable reason leading to prothrombotic effects of oral contraceptive pills.[1] Our results are also in line with those of a Swedish study where Bremme et al. also reported that the levels of protein C inhibitor increased significantly after the use of oral contraceptive pills for 4 months (4.64 to 4.78 mg/l; p-value=0.02).[7]

A UK-based case-control study using hospital admission with a diagnosis of deep venous thrombosis or pulmonary embolism found an odds ratio of 6.4.[12] Two meta-analyses reported that the risk of cerebral venous thrombosis is increased in COC users (relative risk: 15.9, 95% confidence interval [CI]: 6.98–36.2; odds ratio: 5.59, 95% CI: 3.95–7.91).[13] It is clear from more recent literature that deep vein thrombosis will only be confirmed in 25-50% of cases at most where it is clinically suspected.[14]

The present study is first of its kind in local population and adds to the limited already published research evidence on the topic. In this study, persistent use of second generation oral contraceptive for 3 months was found to induce a prothrombotic state by a significant increase in the levels of protein C inhibitor which warrants a change in current practice by promoting the use of other forms of contraceptive measures where possible as well as strict monitoring of coagulation profile in a patient on oral contraceptives so that timely identification and management of hypercoagulable state may improve the outcome

of such cases in future practice.

Conclusion

It can be concluded from the study that the use of second generation oral contraceptive for 3 months induces prothrombotic state by increase in the levels of protein C inhibitor in the blood in the absence of any other prothrombotic conditions. This warrants a change in the current practice of use of the second generation oral contraceptives which should be prescribed after taking detailed history and counselling of the patient about symptoms of clot formation. The coagulation profile of the patient should be monitoring during the use of oral contraceptive for timely identification and management of the hypercoagulable state.

Limitation of the study: A major limitation was that effect of oral contraceptive on other procoagulant and anticoagulant factors was not measured. Another limitation was that the effect of stopping the treatment on normalization of these factors was not evaluated, that could help in determining the safety window and will help in stopping and switching to other contraceptive methods.

REFERENCES

1. Kemmeren JM, Algra A, Meijers JC, Tans G, Bouma BN, Curvers J, et all. Effect of second- and third-generation oral contraceptives on the protein C system in the absence or presence of the factor V Leiden mutation: a randomized trial. Blood. 2004;103(3):927-33. https://doi.org/10.1182/blood-2003-04-1285
2. Özkan D, Akar N. Analysis of Protein C Inhibitor/SERPINA5. Turkish Journal of Clinics and Laboratory 2020;11(1):75-79. https://doi.org/10.18663/tjcl.645392
3. Yang H, Wahlmüller FC, Sarg B, Furtmüller M, Geiger M. A+-helix of protein C inhibitor (PCI) is a cell-penetrating peptide that mediates cell membrane permeation of PCI. J Biol Chem. 2015;290(5):3081-91. https://doi.org/10.1074/jbc.M114.581736
4. Einfinger K, Badrnya S, Furtmüller M, Handschuh D, Lindner H, Geiger M. Phospholipid Binding Protein C Inhibitor (PCI) Is Present on Microparticles Generated In Vitro and In Vivo. PLoS One 2015;10(11):e0143137. https://doi.org/10.1371/journal.pone.0143137
5. Meijers JC, Marquart JA, Bertina RM, Bouma BN, Rosendaal FR. Protein C inhibitor (plasminogen activator inhibitor-3) and the risk of venous thrombosis. Br J Haematol 2002;118(2):604-9. https://doi.org/10.1046/j.1365-2141.2002.03652.x
6. Tans G, Curvers J, Middeldorp S, Thomassen MC, Meijers JC, Prins MH, et all. A randomized cross-over study on the effects of levonorgestrel- and desogestrel-containing oral contraceptives on the anticoagulant pathways. Thromb Haemost 2000;84(1):15-21. https://doi.org/10.1055/s-0037-1613960
7. Bremme K, Hamad RR, Berg E, Strandberg K, Stenflo J. The APC-PCI concentration as an early marker of activation of blood coagulation: a study of women on combined oral contraceptives. Thromb Res 2012;130(4):636-9. https://doi.org/10.1016/j.thromres.2011.11.006
8. Salman ST, Hussein AA, Saadi RK. Effect of Combined Oral Contraception on Coagulation Profiles in Women Attending Fertility Control Clinic in Baqubah City- Iraq. J Fac Med Bagdad 2018;60(1):47-51. https://doi.org/10.32007/jfacmedbagdad.60144
9. Lidegaard Ø, Nielsen LH, Skovlund CW, Skjeldestad FE, Løkkegaard E. Risk of venous thromboembolism from use of oral contraceptives containing different progestogens and oestrogen doses: Danish cohort study. BMJ 2011;343:d6423-d6423. https://doi.org/10.1136/bmj.d6423
10. Brynhildsen J. Combined hormonal contraceptives: prescribing patterns, compliance, and benefits versus risks. Ther Adv Drug Saf 2014;5(5):201-13. https://doi.org/10.1177/2042098614548857
11. Koster T, Small RA, Rosendaal FR, Helmerhorst FM. Oral contraceptives and venous thromboembolism: a quantitative discussion of the uncertainties. J Intern Med 2012;238(1):31-37. https://doi.org/10.1111/j.1365-2796.1995.tb00896.x
12. Vessey MP, Doll R. Investigation of relation between use of oral contraceptives and thromboembolic disease. Br Med J 2016;2(5599):199-205. https://doi.org/10.1136/bmj.2.5599.199
13. Jang YS, Lee ES, Kim YK. Venous thromboembolism associated with combined oral contraceptive use: a single-institution experience. Obstet Gynecol Sci 2021;64(4):337-344. https://doi.org/10.5468/ogs.20374
14. Huisman MV, Büller HR, ten Cate JW, Vreeken J. Serial impedance plethysmography for suspected deep venous thrombosis in outpatients. The Amsterdam General Practitioner Study. N Engl J Med. 2017;314(13):823-8. https://doi.org/10.1056/NEJM198603273141305

CONFLICT OF INTEREST
Authors declare no conflict of interest.
GRANT SUPPORT AND FINANCIAL DISCLOSURE
None declared.